PB86-231867

Ozone Inhibits Prostacyclin Synthesis in Pulmonary Endothelium

North Carolina Univ., Chapel Hill

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. PB86-231867
EPA/600/J-85/423		

4. TITLE AND SUBTITLE
OZONE INHIBITS PROSTACYCLIN SYNTHESIS IN PULMONARY ENDOTHELUIM (Journal Version)

5. REPORT DATE
1985

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
MITCHELL FRIEDMAN

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
DEPARTMENT OF MEDICINE AND RADIOLOGY
CENTER FOR ENVIRONMENTAL MEDICINE
UNC, CHAPEL HILL, NC 27514

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
HEALTH EFFECTS RESEARCH LABORATORY -RTP,NC
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/11

15. SUPPLEMENTARY NOTES
GEORGEM. GOLDSTEIN CRB/ITD/HERL
REF: PROSTAGLANDINS, 30(6):1069-1083, December 1985

16. ABSTRACT
The effects of ozone on lung arachidonate metabolism in-vitro were studied in cultured bovine pulmonary endothelial cells exposed for 2 hours to ozone in concentrations up to 1.0 ppm. A concentration-dependent decrease in prostacyclin synthesis was found (90% decrease at the highest ozone level of 1.0ppm). The inhibition of prostacyclin synthesis was not due to a decreased release of arachidonic acid from membrane lipids. We also examined the gypoxic pulmonary vasoconstrictive response to 10% oxygen inhalation in anesthetized dogs in-vivo after exposure to 1.0 ppm ozone for 1 hour. Pulmonary vascular resistance was significantly increased after ozone exposure, similar to the findings in dogs given indomethacin (15 mg/kg). The percentage change in the hypoxic pulmonary pressor response was similar between the ozone exposure and indomethacin-treated groups, although due to the variance of the pulmonary vascular resistance values during hypoxia the results did not reach statisticla significance. These results suggest that ozone inhalation affects pulmonary endothelial arachidonate metabolism in-vivo as well as in-vitro.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	16
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

OZONE INHIBITS PROSTACYCLIN SYNTHESIS IN PULMONARY ENDOTHELIUM

Mitchell Friedman, Michael C. Madden, D. Stephen Saunders, Kenneth Gammon, Gilbert C. White, II and Lester Kwock

Department of Medicine and Radiology
and Center for Environmental Medicine
University of North Carolina School of Medicine
Chapel Hill, NC 27514

ABSTRACT

The effects of ozone on lung arachidonate metabolism in-vitro were studied in cultured bovine pulmonary endothelial cells exposed for 2 hours to ozone in concentrations up to 1.0 ppm. A concentration-dependent decrease in prostacyclin synthesis was found (90% decrease at the highest ozone level of 1.0 ppm). The inhibition of prostacyclin synthesis was not due to a decreased release of arachidonic acid from membrane lipids. We also examined the hypoxic pulmonary vasoconstrictive response to 10% oxygen inhalation in anesthetized dogs in-vivo after exposure to 1.0 ppm ozone for 1 hour. Pulmonary vascular resistance was significantly increased after ozone exposure, similar to the findings in dogs given indomethacin (15 mg/kg). The percentage change in the hypoxic pulmonary pressor response was similar between the ozone exposure and indomethacin-treated groups, although due to the variance of the pulmonary vascular resistance values during hypoxia the results did not reach statistical significance. These results suggest that ozone inhalation affects pulmonary endothelial arachidonate metabolism in-vivo as well as in-vitro.

INTRODUCTION

Ozone is a major component of photochemical air pollution capable of causing significant lung damage including pulmonary endothelial cell injury and pulmonary edema when inhaled (1). The possible consequences of long-term or repeated exposure to ozone remains a largely unresolved issue with considerable implications for public health. The exact mechanisms of tissue injury by ozone are unknown. It has been shown that ozone can react with unsaturated lipids in cell membranes and organelles to cause ozonolysis at these bonds. Through its reaction with polyunsaturated fatty acids and conversion of lipids to free radicals, ozone may render the cell membrane susceptible to peroxidative attack by oxygen (2).

The lung is a major site of eicosanoid metabolism. These potent autocoids, derived from selective oxidation of arachidonic acid, have been suggested to be involved in the inflammatory response of the lung to ozone (3). Pulmonary endothelial synthesis of prostacyclin, the major arachidonate metabolite, is catalyzed by cyclooxygenase followed by prostacyclin synthetase. Prostacyclin is a known potent inhibitor of platelet aggregation and a pulmonary vasodilator (4).

We report here studies to show the effects of ozone exposure, in-vitro, on arachidonate metabolism of cultured pulmonary endothelial cells. We also studied the effects of ozone exposure in-vivo, in dogs, on pulmonary vascular resistance and the pulmonary vasoconstrictor response to hypoxia and compared these reponses to changes observed after cyclooxygenase inhibition with indomethacin.

METHODS

Cell Culture

Pulmonary artery endothelial cells were harvested by gentle scraping of the intimal surface of the main stem pulmonary arteries of calves (5). The cells were dispersed into 25 cm^2 culture flasks containing Medium 199 supplemented with 10% fetal calf serum, penicillin (100 µg/ml), streptomycin (100 µg/ml), gentamicin (50 µg/ml), thymidine (5 µg/ml) and deoxycytidine (5 µg/ml) and grown to confluence in 5% CO_2, 95% air at 37°C. The cells were subcultured using 0.25% trypsin. Cells from passages 2-17 were used. Endothelial cells were identified by their light and electron microscopic appearance, positive immunofluorescence staining for factor VIII antigen and by the presence of angiotensin converting enzyme.

Smooth muscle cells were grown from explants of the media of main pulmonary arteries of calves (6) in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum and antibiotics (in similar concentration to that used for the endothelial cells). Smooth muscle cells were identified by their light and electron microscopic appearance. The cells were subcultured using 0.25% trypsin and cells from passages 2-10 were studied.

Ozone Exposure System

For the ozone exposure studies, cells were removed by trypsin from the culture flasks and grown to confluence in 100 ml volume glass roller bottles in media supplemented with 50 mM HEPES. Prior to exposure, the cells were washed thoroughly with serum-free medium 199 and 8.0 ml of serum-free Medium 199 with 50 mM HEPES added to the bottles. The roller bottles with confluent cells were placed on a roller apparatus housed within an enclosure

that was maintained at 37°C. An adaptor at the bottle opening allowed gas to continuously enter and leave the bottle, as it rolled. Ozone was continuously generated by ultraviolet irradiation of ultrapure air. The ozonated air was introduced into the bottles at a constant flow of 10 ml/min and the ozone concentration was continuously monitored by chemiluminescence at the bottle inlet and maintained within ±3% of the desired level (Model 350-2R, Meloy Laboratories, Springfield, MA). The ozone analyzer was calibrated before each exposure period using a precision ozone calibration source (Model 1000, Columbia Scientific Industrial Corporation, Houston, TX). Since the bottles are rolling continuously during the ozone exposure period in a small volume of media, most of the attached cells are being directly exposed to the gaseous pollutant at any given time (estimated at 90%). Furthermore, the media is protein-free and buffered by HEPES which maintained pH constant during the exposure period.

Assay for Arachidonate Metabolites

Radioimmunoassay - After exposure of the cells to ozone, the media was removed, centrifuged for 15 min at 2000 x g, and aliquots of the supernatant used for duplicate measurements of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin and of thromboxane B_2 (TxB_2), the stable metabolite of thromboxane A_2 by radioimmunoassay (New England Nuclear Corp., Boston, MA). The cells were removed by trypsin and counted by hemocytometry. The data are reported as ng metabolite/10^6 cells.

For experiments involving addition of exogenous arachidonate, cells were washed twice with serum-free media and 8.0 ml of serum-free media added followed immediately by the addition of arachidonic acid (99% purity, Sigma Chemical Co., St. Louis, MO) dissolved in ethanol (0.6% v/v final concentration).

High Pressure Liquid Chromatography - Cells in roller bottles were washed, incubated for 19 hours with 1 µCi/10^6 cells of ^3H-arachidonic acid (New England Nuclear Corp., Boston, MA), washed twice with serum free media, 8.0 ml of serum free media with 50 mM HEPES added, and the cells exposed to ozone as previously described. After exposure, the media was removed, acidified to pH 3.5 with 10% formic acid and extracted with 2 volumes of cyclohexane/ethyl acetate (1:1). After centrifugation at 1500g for 5 min, the organic layer was removed and the medium extracted with 2 volumes of ethyl acetate. The combined organic phase was dried under a constant stream of N_2 in siliconized glass tubes and stored at -70°C until used. The dried metabolite mixture was resuspended in a minimal

volume of 100% methanol and injected into a HPLC system (Waters Associates, Milford, MA) an Altex ODS 5µm ultrasphere reversed phase column. An acetonitrile/H_2O/phosphoric acid (pH 2.0) gradient, from 30% to 95% acetonitrile, modified from the method of Van Rollins et al was used (7). One minute fractions were collected throughout each HPLC run and radioactivity was quantified by liquid scintillation counting. Individual metabolites were identified by comparison to retention times of authentic arachidonate metabolites.

Animal Studies

Mongrel dogs were anesthetized with pentobarbital (25 mg/kg), intubated with a cuffed Teflon endotracheal tube, and ventilated with a constant volume ventilator (FIO_2 of 0.21). Catheters were placed in the femoral artery, pulmonary artery (Swan-Ganz), and, in some dogs, the left atrium. Cardiac output was measured using a thermodilution technique (Model 701, Instrumentation Laboratory, Lexington, MA). Mean pulmonary artery, pulmonary capillary wedge, and left atrial pressures were measured at end-exhalation by transducers referenced to the mid-chest level (Model 1280C, Hewlett Packard, Waltham, MA). Pulmonary vascular resistance was computed as mean pulmonary artery pressure minus mean pulmonary capillary wedge pressure divided by cardiac output. P_aO_2, P_aCO_2, and pH_a were measured in arterial blood with standard electrodes (Model BM SBMK2, Radiometer, Copenhagen).

Pulmonary vascular resistance and arterial blood gases were serially measured at baseline (FIO_2=0.21), during a 20 min period of hypoxia (FIO_2=0.10), and then for an additional 20 min period of recovery (FIO_2=0.21). The animals were then separated in a random fashion into 3 experimental groups. Eight dogs were infused with indomethacin (15 mg/kg) in phosphate/ethanol buffer and then sham-exposed to 0.0 ppm ozone for one hour. Six additional dogs were infused with the buffer/ethanol vehicle only and then sham-exposed for one hour and served as controls. Seven additional dogs were infused with the vehicle alone and then exposed to 1.0 ppm ozone for one hour.

The dogs were exposed to ozone using a method that has been described in detail previously (8). Briefly, dry filtered air was passed through an ultraviolet light source, diluted with additional air, humidified and warmed to 37°C and delivered to the animal via a one-way valve. During the exposure period, respiration was maintained by transvenous phrenic nerve stimulation. The ozone concentration was continuously monitored at the valve opening and kept within 5% of the desired concentration during the exposure period.

Following the exposure period, the hypoxic challenge protocol was repeated in all dogs.

RESULTS

The effects of a 2 hour exposure to ozone (0.0-1.0 ppm) on production of prostacyclin and thromboxane by cultured pulmonary endothelial cells are shown in Table 1. Exposure to ozone resulted in a significant concentration-dependent reduction of endothelial prostacyclin production and a reduction in thromboxane production. Exposure to the highest ozone concentration (1.0 ppm) decreased prostacyclin production by 90%, $p < 0.05$. Cells exposed to ozone remained attached to the bottle, had a normal morphologic appearance by inverted phase-microscopy and >95% excluded trypan blue after exposure. No systematic difference was noted in the response of early passage cells compared to later passage cells.

Table 1

RADIOIMMUNOASSAY OF THE PRODUCTS OF PROSTACYCLIN (6-KETO-$PGF_{1\alpha}$) AND THROMBOXANE A_2 (TxB_2) IN PULMONARY ENDOTHELIAL CELLS EXPOSED TO OZONE.[a]

ng metabolite/10^6 cells

Ozone Dose(ppm)	0.0	0.1	0.3	1.0
6-keto-$PGF_{1\alpha}$	2.94 ±1.27	1.16 ±0.41	0.60[b] ±0.10	0.30[b] ±0.18
n	7	8	9	6
TxB_2	0.56 ±0.26	0.12 ±0.06	0.07[b] ±0.03	0.03[b] ±0.02
n	6	3	6	6

[a]Endothelial cells were grown in roller bottles and exposed to ozone for 2 hours as described in Methods. The effects of varying concentrations of ozone were compared using cells of the same passage number. The cells were removed from the bottles by trypsin and counted after exposure. The arachidonate metabolites were measured by radioimmunoassay and the data expressed as mean±SEM. Significant differences from the 0.0 ppm ozone value were computed using the Dunnet's least significance difference test; [b]$p < .05$.

When endothelial cells were exposed to exogenous arachidonate (20 μM for 5 min) immediately after exposure, thus bypassing the release step of arachidonate from cell membrane lipid stores, the ozonated cells converted significantly less of this substrate into prostacyclin than did control cells, and again in a dose-dependent fashion (Table 2).

Table 2

EFFECTS OF INCUBATION OF ARACHIDONIC ACID WITH PULMONARY ENDOTHELIUM AFTER OZONE EXPOSURE[a]

Percent of control 6-keto-$PGF_{1\alpha}$ values

Ozone Dose (ppm)	0.03	0.10	0.30	1.00
% of control	111±15	107±28	64±12[b]	36±14[c]
n	7	7	12	7

[a]Endothelial cells were incubated with arachidonic acid (5 μM for 5 min) immediately after a 2 hour exposure to ozone (see Methods). Prostacyclin production was assessed by measuring the amount of 6-keto-$PGF_{1\alpha}$ by radioimmunoassay. The data are expressed as per cent change from the control (0.0 ppm) exposure group mean±SEM. The production of 6-keto-$PGF_{1\alpha}$ in the control exposure group was 377±95 ng/10^6 cells (n=15). Significant differences from the 0.0 ppm value were computed using the Dunnet's least significance test; [b]$p < .05$, [c]$p < .01$.

In order to determine if the effects observed were due to the reaction of ozone with the media itself with production of a toxic intermediate that might inhibit endothelial prostacyclin production, the exposure media alone was exposed to 0.0 or 1.0 ppm ozone for 2 hours. The media was removed following the exposure and incubated for 2 hours with unexposed confluent cultures of endothelial cells and the amount of 6-keto-$PGF_{1\alpha}$ measured. No significant difference in prostacyclin production between the 2 groups was found. These data suggest that the ozone did not react with the media itself to produce a long-lived toxic intermediate that might inhibit prostacyclin production.

To determine if ozone exposure resulted in a decrease in the release of arachidonic acid from lipid stores, endothelial cells were incubated with ^3H-arachidonate for 19 hours, washed thoroughly, incubated with indomethacin (5 μg/ml) for one hour and then exposed to ozone for 2

hours. Release of the labelled arachidonate from the cells was determined by liquid scintillation counting of the media after ozone exposure relative to the counts in the media (after exposure) plus the counts remaining in the cells after removal by 0.25% trypsin. The results are shown in Table 3. Ozone-exposed cells released significantly more radioactivity than sham-exposed cells (approximate 4-fold increase at the 1.0 ppm exposure level) in a concentration-dependent fashion.

Table 3

THE EFFECT OF OZONE EXPOSURE ON ARACHIDONIC ACID RELEASE FROM PULMONARY ENDOTHELIUM[a]

Per cent release of ^{3}H-Arachidonic acid

Ozone Dose (ppm)	% release	n
0.0	0.65±0.12	6
0.3	1.21±0.20[b]	6
1.0	2.21±0.60[c]	6

[a]Endothelial cells were incubated for 19 hours with ^{3}H-arachidonic acid and exposed to ozone for 2 hours. Data are expressed as mean±SEM. Significant differences were computed using the Dunnet's least significant difference test; [b]$p<0.05$, [c]$p<0.01$.

Ozone could affect various enzymes in the arachidonic acid metabolic pathway, e.g. lipoxygenase, cyclooxygenase, thromboxane synthetase or prostacyclin synthetase. A high pressure liquid radiochromatogram of ^{3}H-arachidonic acid conversion products in endothelial cells exposed for 2 hours to 1.0 ppm ozone demonstrated that the reduction in 6-keto-$PGF_{1\alpha}$ was not accompanied by any measurable increase in other cyclooxygenase or lipoxygenase products (data not shown). The amount of ^{3}H-arachidonate was changed after ozone exposure (Table 3). The increase in radioactivity was accounted for as an increase in arachidonic acid and not by increases in other metabolites or in the solvent front.

We also compared the effects of ozone exposure on pulmonary vascular smooth muscle cell production of prostacyclin. Pulmonary smooth muscle cells were exposed to ozone for 2 hours in a similar fashion to the endothelial cells. Since pulmonary smooth muscle cells

synthesize only small quantities of prostacyclin, we incubated the cells with 20 μM arachidonate for 5 minutes immediately after exposure. Ozone exposure resulted in a modest depression of prostacyclin synthesis after ozone exposure (Table 4).

Table 4

EFFECTS OF OZONE EXPOSURE ON PROSTACYCLIN PRODUCTION OF PULMONARY VASCULAR SMOOTH MUSCLE CELLS[a]

	Per cent of control 6-keto-$PGF_{1\alpha}$ values		
Ozone Dose (ppm)	0.1	0.3	1.0
% of control	76±13	55±10[b]	59±11[b]
n	6	6	9

[a]Bovine pulmonary vascular smooth muscle cells attached to the wall of roller bottles were exposed to ozone for 2 hours, washed and immediately incubated with arachidonic acid (20 μM for 5 min). The amount of 6-keto-$PGF_{1\alpha}$ was measured by radioimmunoassay (ng/10^6 cells) and the data expressed as per cent change from control (0.0 ppm) exposure mean±SEM. The production of 6-keto-$PGF_{1\alpha}$ in the control exposure group was 11(3) ng/10^6 cells (n=9). Significant differences were computed using the Dunnet's least significant difference test; [b]$p<0.01$.

These data demonstrate that ozone exposure in-vitro inhibits prostacyclin production and thromboxane production in cultured pulmonary vascular cells. This inhibition was not due to a decrease in arachidonic acid release. These data suggest inactivation of cyclooxygenase and/or prostacyclin synthetase enzyme activity.

Since it has been suggested that the pulmonary vasoconstrictor response to hypoxia may be modulated by cyclooxygenase, we studied the in-vivo effects of a 1 hour exposure in dogs to 1.0 ppm ozone on pulmonary hemodynamics including the pulmonary vascular response to hypoxia. We also compared this response to that of dogs who had been pretreated with a known cyclooxygenase inhibitor, indomethacin. The data are shown in Figure 1.

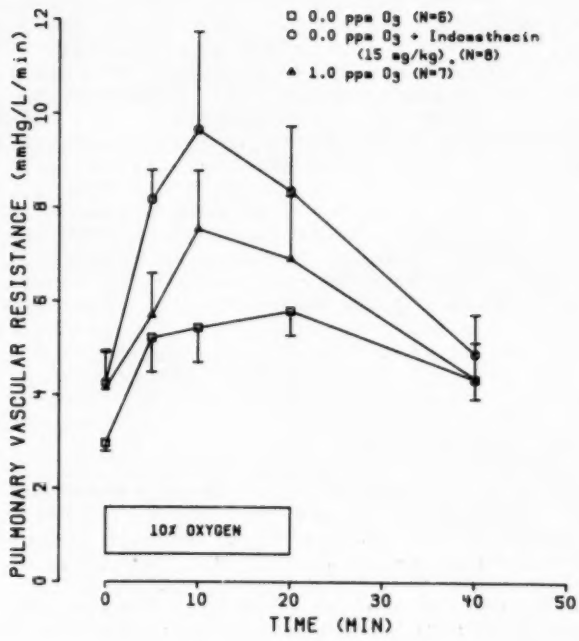

Figure 1. Effects of a 20 minute exposure to 10% O_2 on pulmonary vascular resistance (see Methods) in air-exposed dogs pretreated with indomethacin, control dogs (air exposure and no indomethacin) and dogs exposed to 1.0 ppm ozone for one hour. Data shown as mean ± SEM.

The control dogs demonstrated a time-dependent increase in pulmonary vascular resistance during hypoxia.

Small and non-significant decreases in cardiac output (5%), P_aCO_2 (4%) and pH_a (<1%) and a significant decrease in P_aO_2 (50%) occurred during the hypoxic period.

All three groups had similar pre-exposure hemodynamic values and similar values for arterial blood gases and cardiac output during both the pre-exposure and post-exposure hypoxic challenge periods. No pulmonary edema developed in any group of dogs as determined by post-mortem determinations of gravimetric lung water.

Dogs pretreated with indomethacin and exposed to air, had a significant increase in baseline (normoxic) pulmonary vascular resistance, compared to the pre-exposure values (4.26 vs. 2.83 mmHg/L/min respectively, $p < 0.05$). Exposure to ozone resulted in a similar significant ($p<0.05$) increase in pulmonary vascular resistance compared to pre-exposure values. The increase in pulmonary vascular resistance after exposure to ozone was not significantly different from the values in the dogs treated with indomethacin and exposed to air. The pulmonary vascular resistance values in both groups (ozone exposure and indomethacin treatment) were both significantly increased compared to the post-exposure values of the control dogs.

During the 20 minute hypoxic challenge period, a significant ($p < 0.05$) augmentation of pulmonary vascular resistance, compared to air-exposed control animals, occurred in the indomethacin treated animals (Figure 1). A rather similar response to the hypoxic challenge was found in the dogs exposed to 1.0 ppm ozone. Although the percentage change was similar between these groups, due to the variance of the pulmonary vascular resistance values during hypoxia, the results did not reach statistical significance.

DISCUSSION

We have demonstrated a dose-dependent inhibition of prostacyclin and thromboxane synthesis in endothelial cells exposed to ozone. The data presented (Tables 2 and 3) supports the hypothesis that a decrease in cyclooxygenase and/or prostacyclin synthetase activity may have been induced by ozone exposure. The possible relationships between arachidonate metabolism and other cellular events which may be induced by ozone include formation of free radical reactions, lipid peroxidation and peroxidative product activity. It is not possible from the present experiments to differentiate a direct effect of ozone from an indirect effect, e.g. formation of a toxic intermediate. Cyclooxygenase activity has been shown to be inhibited by "high" concentrations of lipid peroxides (16). Ozone could react with cell membranes

resulting in peroxidation of arachidonic acid (3,17). Competitive inhibition of cyclooxygenase could also have occured due to an increased release of other unsaturated fatty acids, e.g. linoleic acid (18,19). The cyclooxygenase enzyme might also be sensitive to sulfhydryl oxidation induced by ozone (20). A decrease in prostacyclin synthetase activity in the presence of various lipid peroxides and hydroperoxides has been demonstrated (21,22).

Ozone exposure could also have resulted in the production of various reactive intermediates of oxygen that may have been responsible for the decrease in endothelial cell prostacyclin production. Exposure of the same endothelial cell lines to gamma irradiation (0-3000 rads) resulted in a dose-dependent increase in prostacyclin production (3-fold increase at the 3000 rad level)(23). Oxygen radicals generated by the xanthine-xanthine oxidase system have also been demonstrated to result in an increase in endothelial cell prostacyclin production (24). The major oxidant species produced in both of these studies were most likely the superoxide and hydroxyl radicals. A decreased prostacyclin production in endothelial cells has been reported after hydrogen peroxide or lipid peroxide exposures (25-26).

The ability to isolate and propagate in culture pure lines of pulmonary endothelial cells now allows studies of specific cellular biochemical and metabolic functions. There are several published methods that allow cells to be exposed to ozone in-vitro. These include exposure through media-filled culture dishes, use of inverted culture flasks, diffusion through teflon membranes or replacement of the incubator atmosphere (9-12). The roller bottle system, used in the present study, allows for essentially direct exposure of the cells to the gas phase of the toxicant (i.e. minimal volume of bathing media and continuous rolling) while maintaining cell viability. Due to the complexity of the delivery system in-vivo including the reactivity of the oxidant pollutant in the airways and the dependence of the delivered dose on ventilation (13), it is difficult to predict the exact concentration of ozone that would be delivered to the pulmonary vessels under various conditions of human exposure. Kobayashi has demonstrated a decrease of prostacyclin-synthesizing activity in rat whole lung homogenates prepared following in-vivo exposure to 0.4 ppm ozone for 24 hours (14). However, these exposures resulted in pulmonary edema formation in the animals. The observed decrease in prostacyclin synthesis may therefore have been overestimated due to the increased amount of lung water in the lung homogenates. There is also evidence in rats

suggesting that lung prostaglandin formation may be altered after ozone exposure to 3.0 ppm ozone for 10 minutes (15).

Alveolar hypoxia results in pulmonary vasoconstriction in most animal species and it has been suggested that arachidonic acid products may modulate this response (27). The hypothesis that prostacyclin may be a modulator of the hypoxic pressor response is supported by the observation that prostacyclin synthesis is increased in canine lung homogenates or isolated rat lung preparations exposed to low concentrations of oxygen (28-29). There is conflicting evidence on the effects of cyclooxygenase inhibitors on pulmonary vascular tone. Studies in intact animals or in isolated perfused lung preparations, using doses of indomethacin of 5 mg/kg or greater, have demonstrated increases in pulmonary vascular resistance similar to the findings in the present study (30-32). The dose of indomethacin used in the present study (15 mg/kg) most likely caused a complete inhibition of cyclooxygenase (32). Indomethacin could also have caused nonspecific effects on enzyme systems other than cyclooxygenase. A decrease in local production of vasodilator prostaglandins (e.g., prostacyclin) appears to be a possible explanation for the observed results seen following ozone exposure (increased pulmonary vascular resistance and a trend toward augmentation of the hypoxic pulmonary pressor response). The demonstration of a similar effect of ozone in-vitro in pulmonary endothelial cells further supports the relevance of the in-vivo data.

The results presented in this report support the hypothesis that ozone can alter arachidonate acid metabolism in the lung. Further studies directed at examining the specific effects of ozone on the arachidonic acid enzymatic pathways in endothelial and other lung cells should allow for a better assessment of the risks posed by this pollutant.

ACKNOWLEDGEMENTS

Supported in part by USEPA grant CR807392, NIH grant HL-29780 and a Parker B. Francis Fellowship. We wish to thank Mrs. Harriette P. Nichols for her expert technical assistance and Dr. Philip A. Bromberg and Dr. Thomas E. Eling for critically reviewing this manuscript.

REFERENCES

1. National Research Council. Ozone and Other Photochemical Oxidants. National Academy of Sciences, 1977. p. 323.

2. Pryor, W. A., J. P. Stanley, E. Blair, and G. B. Cullen. Autoxidation of Polyunsaturated Fatty Acids. Arch. Environ. Health. 28:201. 1976.
3. Roycroft, J. H., W. B. Gunter, and D. B. Menzel. Ozone Toxicity: Hormone-Like Oxidation Products From Arachidonic Acid by Ozone-Catalyzed Autoxidation. Toxicology Letters 1:75. 1977).
4. Moncada, S. and J. R. Vane In:Prostacyclin. (J. R. Vane and S. Bergstrom, eds.) Raven Press, New York, 1979. p. 5.
5. Ryan, U. S. In:Biology of Endothelial Cells (E. A. Jaffe, ed.) Martinus Nijhoff, Boston, 1984. p. 34.
6. Kocan, R. M., N. S. Moss, and E. P. Benditt In:Methods in Cell Biology Vol 21 (C. A. Harris, B. F. Trump, and G. D. Stoner, eds.) Academic Press, New York, 1980. p. 153.
7. Van Rollins, M., M. I. Aveldano, H. W. Sprecher, and L. A. Horrocks, In:Methods in Enzymology (W. E. M. Lands and W. L. Smith, eds.) Academic Press, New York, 1982. p. 518.
8. Friedman, M., J. M. Gallo, H. P. Nichols, and P. A Bromberg. Changes in Inert Gas Rebreathing Parameters After Ozone Exposure in Dogs. Am. Rev. Respir. Dis. 128:851. 1983.
9. Baker, F. D. and C. F. Tumasonis. Modified Roller Drum Apparatus for Analyzing Effects of Pollutant Gases on Tissue Culture Systems. Atmospheric Environment. 5:891. 1971.
10. Alink, G. M., R. M. DeBoer, J. Mol, and J. H. M. Temmink. Toxic Effects of Ozone on Human Cells In Vitro, Exposed by Gas Diffusion Through Teflon Film. Toxicology. 17:209. 1980.
11. Wenzel, D. G. and D. L. Morgan. Role of In Vitro Factors in Ozone Toxicity for Cultured Rat Lung Fibroblasts. Drug and Chemical Toxicology. 5:201. 1982.
12. Samuelsen, G. S., R. E. Rasmussen, B. K. Nair, and T. T. Crocker. Novel Culture and Exposure System for Measurement of Effects of Airborne Pollutants on Mammalian Cells, Environmental Science and Technology. 12:426. 1978.
13. Miller, F. J., D. B. Menzel, and D. L. Coffin. Similarity between Man and Laboratory Animals in Regional Pulmonary Deposition of Ozone. Environmental Res, 17:84. 1978.
14. Kobayashi. T. Effect of Ozone Exposure on Prostacyclin Synthesis in Lung. Prostaglandins. 26:1021. 1983.
15. Menzel, D. B., Anderson, W. G., and Abou-Donia, M. B.. Ozone Exposure Modifies Prostaglandin Biosynthesis in Perfused Rat Lungs. Res. Comm. Chem. Path. Pharm. 15:135. 1976.
16. Warso, M. A. and W. E. Lands. Lipid peroxidation in relation to Prostacyclin and Thromboxane Physiology and Pathophysiology. Brit. Med. Bull. 39:277. 1983.

17. Roehm, J. N., J. G. Hadley, and D. B. Menzel. Oxidation of Unsaturated Fatty Acids by Oxone and Nitogen Dioxide. Arch. Environ. Health. 23:142. 1971.
18. Pace-Asciak, C. and L. S. Wolfe. Inhibition of Prostaglandin Synthesis by Oleic, Linoleic, and Linolenic Acids. Biochim. Biophys. Acta. 152:784. 1968.
19. Kaduce, T. L., A. A. Spector, and R. S. Bar. Linoleic Acid Metabolism and Prostaglandin Production by Cultured Bovine Pulmonary Artery Endothelial Cells. Arteriosclerosis 2:380. 1982.
20. Freeman, B. A. and J. B. Mudd. Reaction of Ozone with Sulfhydryls of Human Erythrocytes. Arch. Biochem. Biophys. 208:212. 1981.
21. Turk, J., A. Wycke, and P. Needleman. Inactivation of Vascular Prostacyclin Synthetase by Platelet Lipoxygenase Products. Biochem. Biophys. Res. Comm. 95:1628. 1980.
22. Reed, G. A., I. O. Griffin, and T. E. Eling. Inactivation of Prostaglandin H synthase and Prostacyclin Synthase by Phenylbutazone: Requirement for Peroxidative Metabolism. Mol. Pharmacol. 27:109. 1985.
23. Saunders, D. S., L. Kwock, and M. Friedman. Effects of Oxidants on Prostacyclin (PGI_2) synthesis and Alpha-Aminoisobutyric Acid (AIB) Transport in Cultured Pulmonary Endothelium (PE). Fed. Proc. 43:808. 1984.
24. Shasby, D. M., J. M. McCormick, S. S. Shasby, and J. C. Goldsmith. Prostacyclin Release from Endothelium Exposed to Oxygen Radicals. Clin. Res. 31:746A. 1983.
25. Moncada, S., R. J. Gryglewski, S. Bunting, and J. R. Vane. A Lipid Peroxide Inhibits The Enzyme in Blood Vessel Microsomes That Generates from Prostaglandin Endoperoxides The Substance (Prostaglandin X) Which Prevents Platelet Aggregation. Prostaglandins. 12:715. 1976.
26. Yamaja-Setty, B. N., E. Jurek, C. Ganley, and M. J. Stuart. Effects of Hydrogen Peroxide on Vascular Arachidonic Acid Metabolism. Prostaglandins Leukotrienes and Medicine 14:205. 1984.
27. Weir, E. K. In:Pulmonary Hypertension (E. K. Weir and J. T. Reeves, eds.) Futura Publishing Co., Mount Kisco, 1984. p. 251.
28. Hamasaki, Y., H-H. Tai, and S. I. Said. Hypoxia Stimulates Prostacyclin Generation by Dog Lung In Vitro. Prostaglandins Leukotrienes and Medicine. 8:311. 1982.
29. Voelkel, N. F., J. G. Gerber, I. F. McMurtry, A. S. Nies, and J. T. Reeves. Release of Vasodilator Prostaglandin, PGI_2, from Isolated Rat Lung During Vasoconstriction. Circ. Res. 48:207. 1981.
30. Kadowitz, P. J., B. M. Chapnick, P. D. Joiner, and A. L. Hyman. Influence of Inhibitors of Prostaglandin Synthesis on the Canine Pulmonary Vascular Bed. Am. J. Physiol. 229:941. 1975.

31. Rubin, L. J., J. D. Highes, and J. D. Lazar. The Effects of Eicosanoid Synthesis Inhibitors on Normoxic and Hypoxic Pulmonary Vascular Tone in Dogs. Am. Rev. Respir. Dis. 132:93. 1985.
32. Hales, C. A., E. T. Rouse, and J. L. Slate. Influence of Aspirin and Indomethacin on Variability of Alveolar Hypoxic Vasoconstriction. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45:33. 1978.

Editor: P.W. Ramwell Received: 9-11-85 Accepted: 10-17-85

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.